

## **Arteriovenous Patterns in Beaked Whales**

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### **LONG-TERM GOALS**

There were two discrete research goals:

- 1) To provide a clear picture of the vascular anatomy in beaked whale heads that will enhance our understanding of the basic biology of beaked whales and act as a baseline from which future morphological (e.g. acoustic pathways), pathophysiological (e.g. decompression sickness and embolus formation) and mathematical modeling (e.g. diving nitrogen kinetics) research can evolve. To conduct a preliminary histologic examination of tissues associated with the extramandibular fat body (EMFB), intramandibular fat body (IMFB) and pterygoid venous lake.
- 2) To describe the vascular morphology of the pulmonary system in bottlenose dolphins and investigate the presence of anatomic intrapulmonary shunts. Results may help inform future models of pulmonary shunting used for mathematical modeling of diving nitrogen saturation levels in cetaceans.

### **OBJECTIVES**

The first objective of the research has been completed. The objective was to describe and better understand the gross morphology of the blood vessels in the heads of beaked whales. Gross anatomical findings and contrast angiographic imaging obtained during the first year of the research were to be supplemented with microscopic examination of vascular structures of interest (e.g. pterygoid venous lake and intramandibular fat body). The final products were to be published in a peer-reviewed scientific journal and presented at the Society for Marine Mammalogy 20<sup>th</sup> Biennial Marine Mammal Conference, Dunedin, NZ.

The second objective of the research was to investigate the vascular morphology within the lungs of bottlenose dolphins (and beaked whales if specimens allowed). The purpose of this investigation was to determine the presence or absence of anatomic intrapulmonary shunts as well as to examine the branching patterns of the pulmonary vasculature.

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## **APPROACH**

The approach for the first objective was unchanged from the original report and primarily involved completion of research and publication goals. Completion of research goals required collection and processing of histologic samples from the pterygoid sinus region and the mandibular acoustic fat bodies. Histologic processing involved staining of tissue slides with hematoxylin and eosin as well as Masson's trichrome. To complete histologic processing a high quality beaked whale specimen was required. Completion of image data post-processing was also necessary.

The second objective required the acquisition of high quality lungs specimens from bottlenose dolphins (and beaked whales when possible). Lungs specimens were to receive traditional anatomical preparation for vascular corrosion casting, involving flushing of blood clots from the vascular system and injection of casting compound followed by corrosion of soft tissue. Resulting vascular casts were to be imaged via scanning electron microscopy (SEM) and micro-computed tomography (microCT).

## **WORK COMPLETED**

### **Objective 1**

The PI and assistant completed all post-processing of data obtained through contrast angiographic CT and gross dissection of all beaked whales specimens. Histologic processing and examination were successfully carried out on tissues obtained from a recent beaked whale specimen of high quality.

The specimen was obtained and tissues were collected from the sinus and acoustic fat body regions of the head. Tissues were fixed in 10% neutral buffered formalin and processed using standard histologic techniques. Duplicate slides were created from the resulting tissue blocks. The first set of slides was stained using hematoxylin and eosin, while the second set was stained with Masson's trichrome for connective tissue differentiation.

Owing to the large number of findings resulting from this research, the PI and assistant generated two separate manuscripts describing their findings. Both manuscripts are currently under informal peer review prior to submission for publication. An oral presentation of the findings was delivered at the 2013 SMM (Society for Marine Mammalogy) Biennial Marine Mammal Conference in Dunedin, NZ.

### **Objective 2**

The second objective of the research was begun but has encountered significant delays. Due to a prolonged and geographically extensive morbillivirus mortality event plaguing bottlenose dolphins along the East coast of the U.S., quality lung specimens were not accessible. Primary tissue targets for morbillivirus infections are the lungs and central nervous system. Pulmonary sequelae observed in specimens commonly involved severe pulmonary congestion, bronchopneumonia and moderate to severe diffuse to coalescing, multifocal emphysematous bullae. Vascular congestion often resulted in extensive blood clotting of pulmonary vasculature. These pulmonary pathologies rendered all fresh specimens obtained unusable. Due to these complications, a no-cost extension was requested to facilitate continuation of the research to completion.

A single unaffected left lung from a bottlenose dolphin was obtained and used to test the vascular casting and imaging methodology. Balloon catheters were inserted into the proximal pulmonary arteries and veins of the lung and a single large catheter was inserted into the primary bronchus. A large syringe was used to slowly inflate the lung and remove any regions of atelectasis before

perfusing it with 0.9% phosphate buffered saline to flush out frank and clotted blood. Once the effluent ran clear, the pulmonary vessels were allowed to drain. The lung was then floated in a water bath and two separate mixtures of methyl-methacrylate (Mercox™ casting compound, Ladd Research) were prepared for injection into the pulmonary arteries and veins. A single 60mL syringe of blue acrylic was injected into the pulmonary vein, followed by simultaneous injections of blue and red acrylic into the pulmonary veins and arteries, respectively. Following a curing time of 3 hours, the lung was placed into an aqueous solution of 15% potassium hydroxide (KOH) to begin the corrosion process. Approximately 3 weeks later, the vascular casts were removed from the KOH and rinsed with vinegar and water and allowed to air dry. After examination of various vascular segments under a dissecting microscope to locate good microvascular casts, the specimens were imaged via micro-computed tomography (microCT) at the Duke Shared Materials Instrumentation Facility (SMIF) Imaging Lab. Post-processing of microCT data was conducted to examine the vascular casts and judge success of capillary injection.

## **RESULTS**

### **Objective 1**

The investigation of the extracranial arterial and venous morphology in the head of beaked whales was successfully completed. Completion involved examination of histologic tissue samples from nasal passages, the pterygoid venous lake and the intramandibular and extramandibular fat bodies. Gross and microscopic examination of the tissues lining the bony nares showed characteristics consistent with vascular erectile tissue, strikingly similar to the morphology of the corpus cavernosum of the mammalian penis. Connective tissue lining the nasal passage is invested with an extensive arteriovenous plexus (Figure 1). Histologic examination of tissues from the EMFB, IMFB and pterygoid venous lake showed surprisingly thin venous walls despite the relatively large venous lumens (Figures 2 through 3). Venous walls were often only a few microns thick—a characteristic of capillaries and post-capillary venules--while the veins themselves could reach diameters in excess of several hundred microns. This finding in conjunction with the apparent robust arterial supply to the tissues suggests that there may be a minimal barrier to the exchange of nitrogen and other gases between the arteries, veins and adipocytes. The thin venous walls may play an important role in nitrogen gas removal from adipocytes during a dive, as well as nitrogen gas absorption by the adipocytes due to retrograde venous blood flow. If indeed nitrogen exchange is facilitated in these tissues, they may have the potential to become supersaturated with nitrogen and subsequently form gas emboli. Additionally, the thin venous walls and their surrounding adipocytes may be easily damaged from expansion of gas emboli and allow for intravascular introduction of fat emboli. Future research should focus on the vascular interface with the adipocytes as well as quantifying such things as microvascular density (e.g. density of capillaries and thin walled veins). Such information could aid in understanding the dynamics of nitrogen gas exchange within those important tissues and shed light on the potential formation of nitrogen gas emboli.

### **Objective 2**

The vascular corrosion cast (Figures 4 & 5) from the single obtained bottlenose dolphin lung provided valuable insights regarding the vascular casting and imaging procedure. Most notably, although some arteriovenous cross over was observed--as evidenced by mixing of blue and red acrylic--the casting results showed a paucity of capillary injection suggesting the need for procedural changes (Figures 6 & 7). Additionally, due to the lack of significant capillary cross over, arteriovenous vascular beds did not remain attached together during the corrosion process, making examination of adjacent vascular beds impossible. As such, future injections will be conducted using a slightly diluted casting compound to

reduce viscosity and improve microvascular perfusion and casting. Additionally, injections will be conducted via only the pulmonary arterial system (no venous injection) in an attempt to maintain anterograde flow of the casting compound and limit antagonistic pressurization of the capillary beds between the arterial and venous systems. These procedural changes should result in higher quality injections that include significant casting of capillary beds.

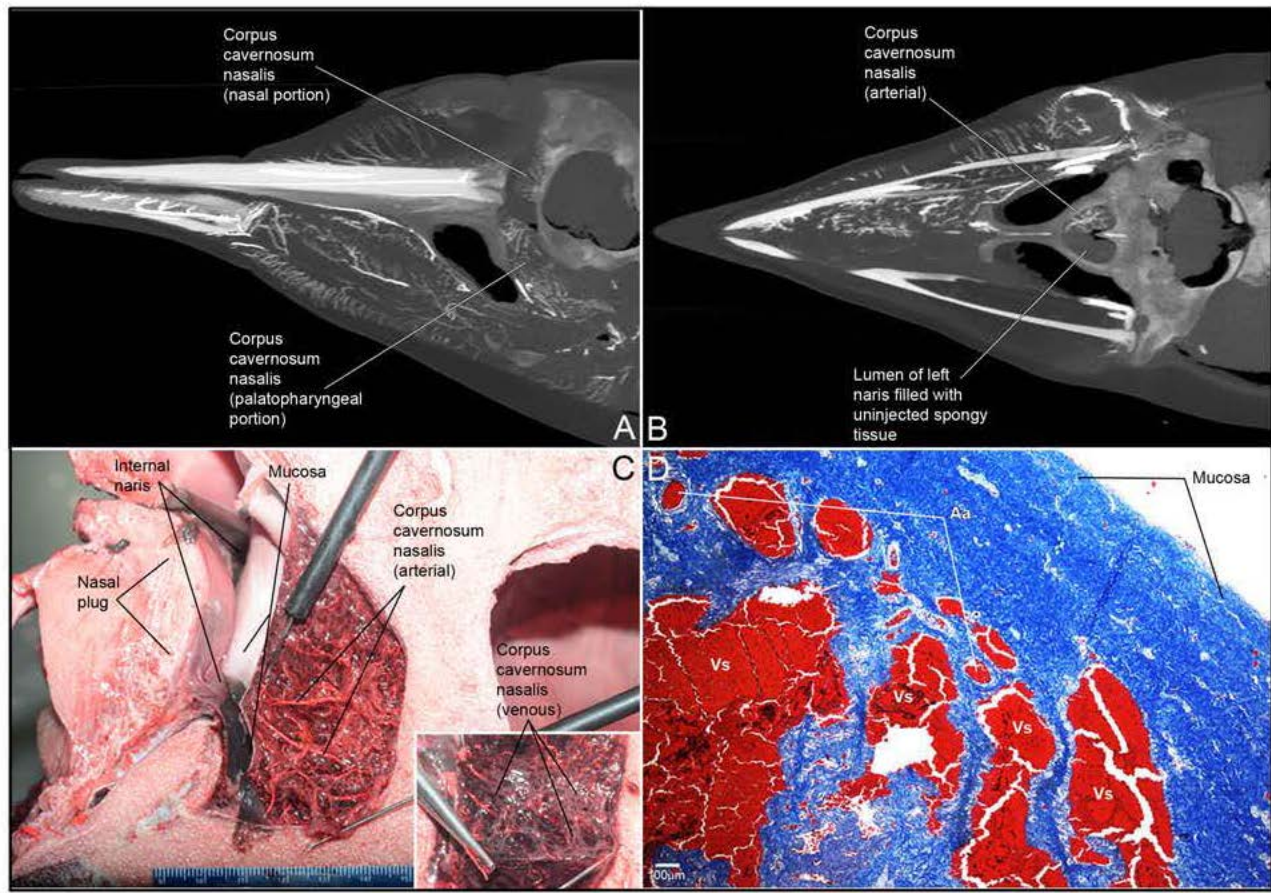
## **IMPACT/APPLICATIONS**

The histologic findings within the head of beaked whales highlighted important characteristics of the tissue interfaces between the vascular system and the targeted areas of interest (e.g. pterygoid air sinuses and IMFB). The exceedingly thin tissue interfaces suggest that exchange of diving nitrogen gas may be possible between the vascular system and connective tissue (e.g. adipocytes). Since adipose tissue is believed to be capable of absorbing relatively larger volumes of nitrogen than other tissues, this finding warrants further research into the morphological (e.g. ultrastructural) and physiological (e.g. gas exchange and nitrogen saturation) characterization of these tissues. Additionally, the thin barrier between the venous system and the lipid-associated tissues in the pterygoid and intramandibular regions suggests a morphology conducive to allowing traumatic intravascular introduction of lipid that can form fat emboli. This finding highlights the need for detailed examination of the pterygoid and IMFB regions in future beaked whale strandings associated with sonar deployment. Our work should help guide future research goals regarding the pathogenesis of emboli in beaked whales believed to be stranded due to decompression sickness and may help refined mathematical models of nitrogen exchange within the tissues.

Owing to the preliminary nature of the pulmonary research, its impact cannot yet be assessed.

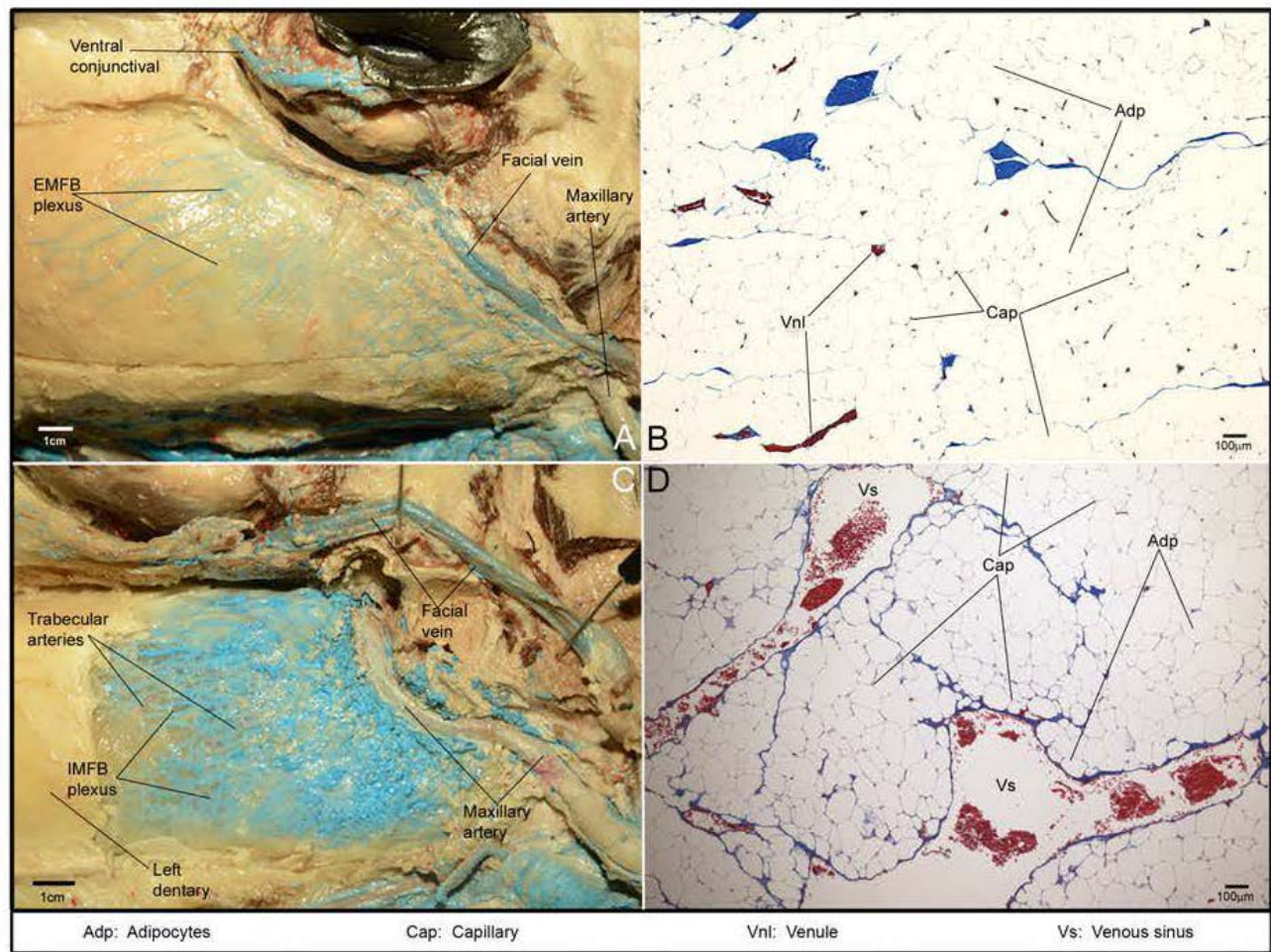
## **RELATED PROJECTS**

A grant proposal was submitted requesting funds to study the histologic, ultrastructural and microvascular details of some of the vascular regions which this current study found to have potentially important functional and pathological implications. The grant proposal was not funded but is awaiting further consideration.

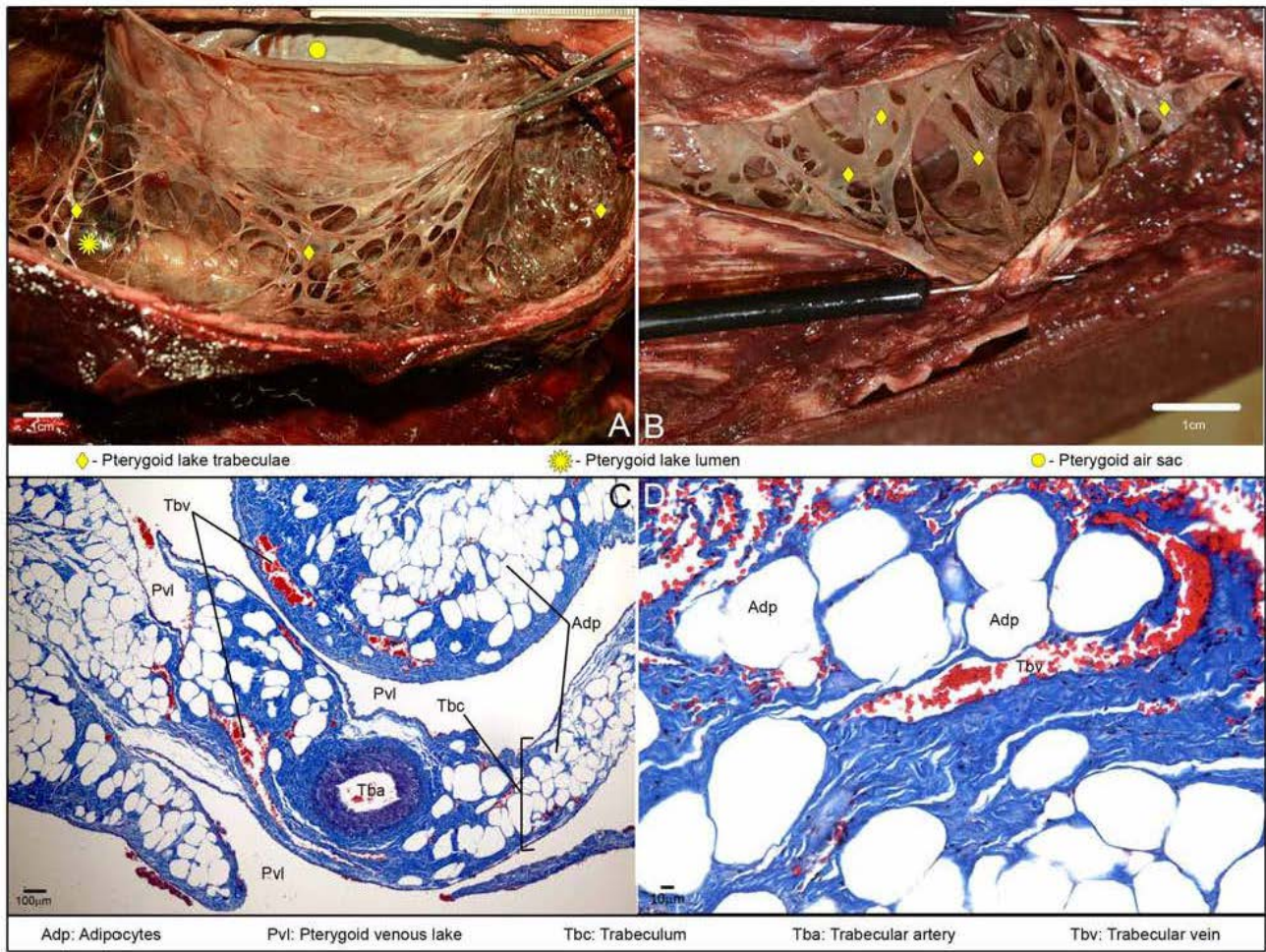


**Figure 1: Computed tomographic (CT) images (sagittal and coronal) showing anatomic locations of corpus cavernosum nasalis (1A & 1B). Gross (1C) and histologic (1D) views of corpus cavernosum nasalis.**



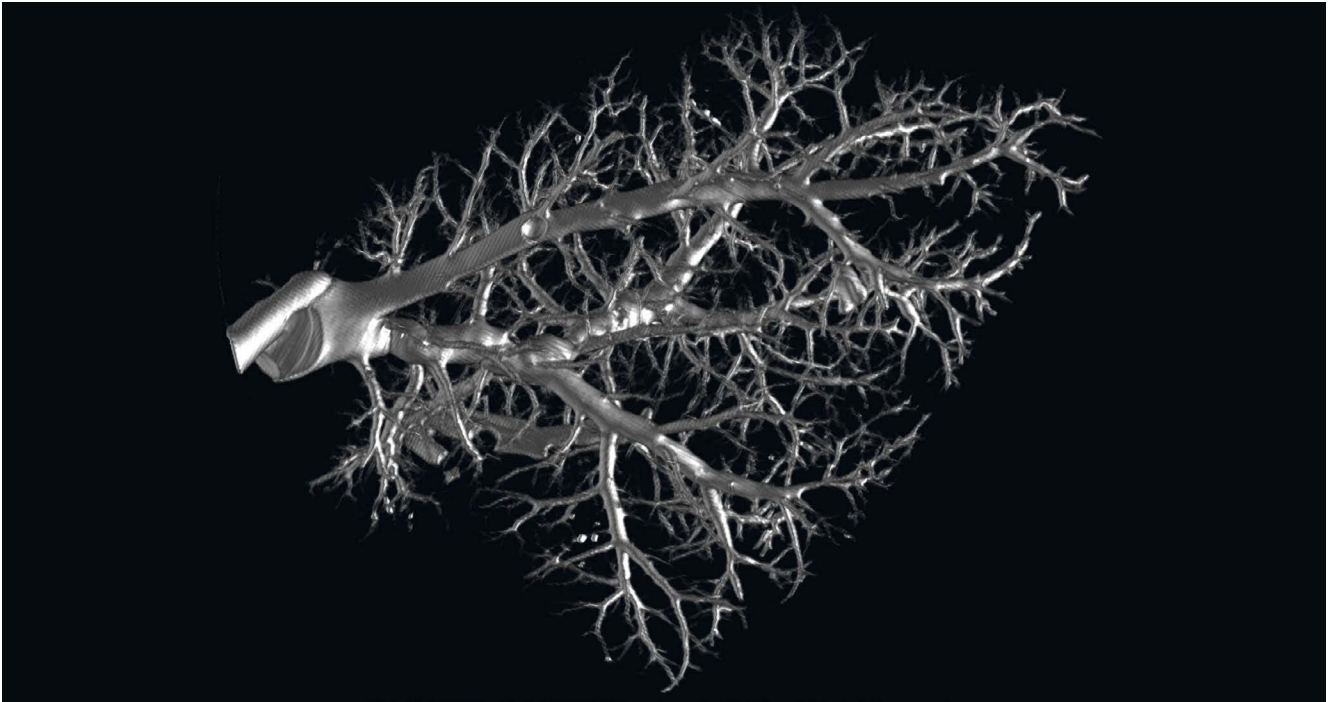


**Figure 2: Gross and histologic appearance of extramandibular fat body (EMFB) and EMFB plexus (2A & 2B). Gross and histologic appearance of intramandibular fat body (IMFB) and IMFB plexus (2C & 2D).**



**Figure 3: Gross appearance of pterygoid venous lake showing un-injected venous lake with dense trabecular distribution (3A & 3B). Histologic appearance of pterygoid venous lake trabeculae showing juxtaposition of adipocytes with arterial and venous structures (3C & 3D). Note thin wall separating adipocytes from venous sinusoids and pterygoid venous lake.**

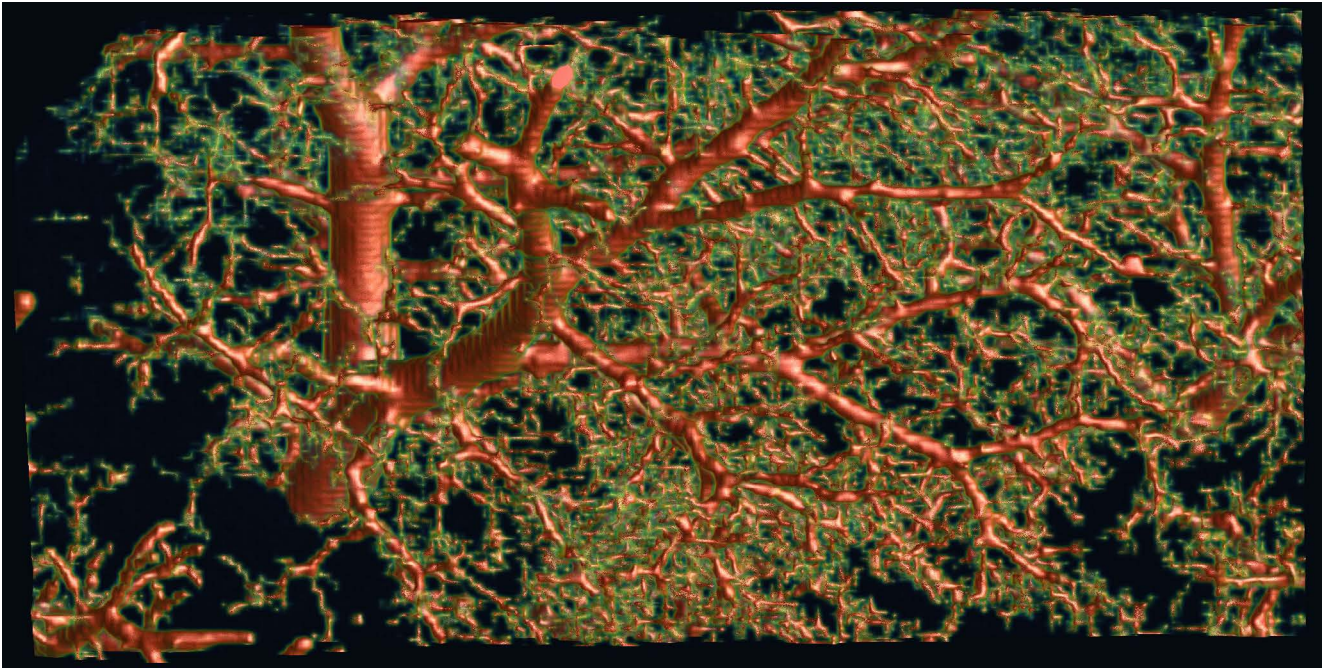




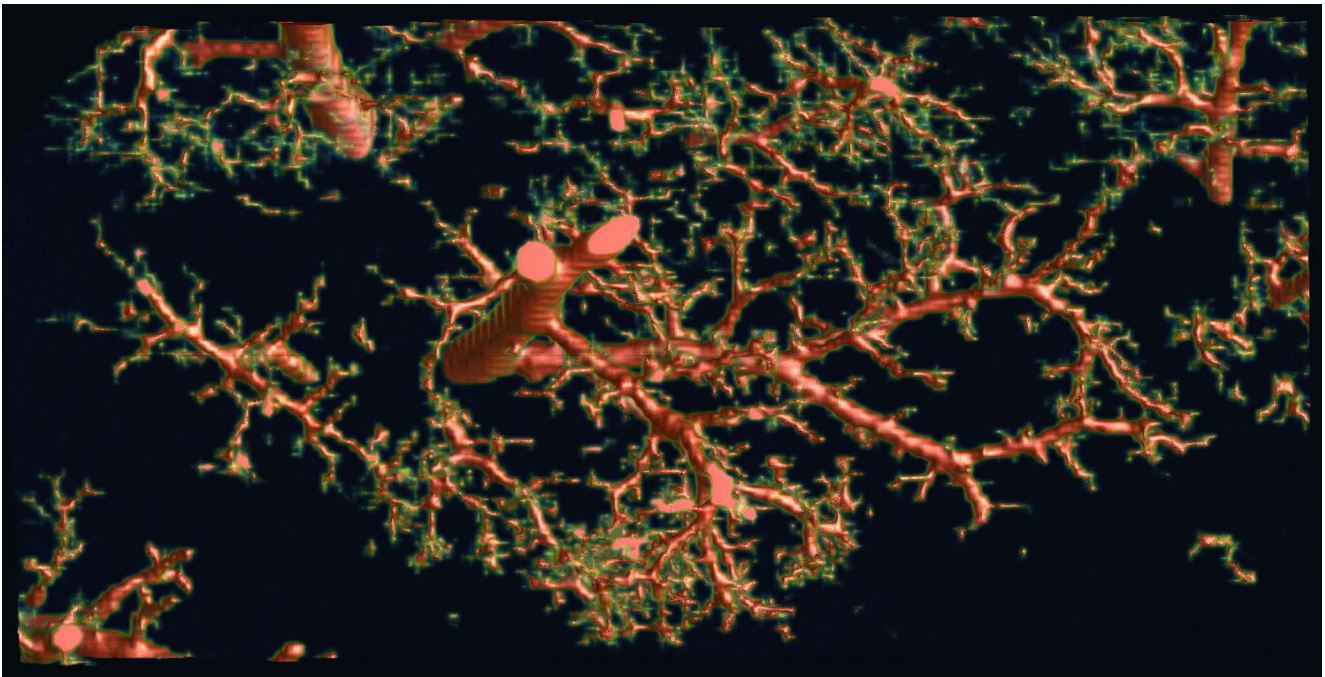
*Figure 4: Volume rendering of a segment of the pulmonary venous tree visualized via microcomputed tomography (microCT). Rendering threshold used (576-1267HU) filtered out smallest venules to reveal main venous trunks.*



*Figure 5: Volume rendering of a segment of the pulmonary venous tree visualized under microcomputed tomography (microCT). Rendering threshold used (259-1036HU) shows complexity of small venules branching off of main venous trunks.*



*Figure 6: Volume rendering from high resolution micro-computed tomographic (microCT) imaging of a pulmonary venous vascular cast from the distal portion of a bottlenose dolphin lung showing high density of pulmonary venules.*



*Figure 7: Volume rendering of a portion of the vascular cast imaged in figure 6. Clipping planes have been employed in order to remove branch overlap and show only a few planes of the vascular tree. Note the abrupt termination of most branches suggesting lack of capillary perfusion.*